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QUALITY OF FRUIT AND FRUIT JUICE IRRADIATED FOR CONTROL OF FOODBORNE PATHOGENS

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ABSTRACT

Exposure to ionizing radiation sterilizes insects, reduces spoilage, and inactivates foodborne pathogens in fruit and fruit products. Irradiation at doses sufficient to meet quarantine requirements did not significantly affect texture, acidity, or soluble solids content of 'Gala' apple fruit, and only temporarily reduces production of some volatile compounds. Irradiation at doses reducing decay and inactivating foodborne pathogens promoted fruit softening, loss of acidity and reduced production of aroma compounds. Combinations of irradiation with an inhibitor of ethylene action, 1-methylcyclopropene, and proper storage temperature management reduced the losses in firmness and acidity, and development of irradiation injury. Irradiation promoted the conversion of ascorbic acid (AA) to dehydroascorbic acid (DHA) in orange juice. The loss of total AA (TAA:AA plus DHA) caused by irradiation was much smaller than that of AA. Based on these results, we estimate that at a 5D dose for *Salmonella,* TAA loss would be 16.6%. Other antioxidants, pH, Brix or concentration of aroma compounds in orange juice would not be affected significantly by a 5D dose of gamma radiation. Among the volatile compounds detected in orange juice, only acyclic monoterpenes were reduced by irradiation.

INTRODUCTION

Exposure to ionizing radiation inhibits ripening and senescence of many fruits, and reduces spoilage and prolongs shelf life of fruit and fruit products (Thomas, 1986). Commercial use of irradiation technology on fruit and fruit products has been limited due to its adverse effects on quality and availability of more economic alternative technologies (Maxie and Abdel-Kader, 1966; Maxie et al., 1971). In recent years, interest in the use of irradiation has increased due to its effectiveness for insect disinfestation (Hallman, 1999) and enhanced food safety (Thayer and Rojkowski, 1999). Irradiation has been approved by the USDA and FDA for use on fruits and vegetables at doses up to 1.0 kGy for insect disinfestation and shelf-life extension. A petition filed by a coalition led by the National Food Processors Association proposes that use of ionizing irradiation up to 4.5 kGy be allowed for control of foodborne pathogens and extension of shelf-life in fresh fruits and their products.

Consumption of fresh fruit and fruit juice has been linked to outbreaks of foodborne illness associated with *E. coli* O157:H7 and *Salmonella* (CDC, 1997; Beuchat, 1997; Parish, 1998). Although many irradiation studies have been conducted on fruit and fruit products for the purpose of ripening inhibition, spoilage reduction and sterilization, there are only a few reports of irradiation research directed toward controlling foodborne pathogens. Some of the major concerns on irradiated fruit and their products include undesirable texture change, development of off-flavor and loss of nutritive value. The objectives of these studies are to investigate the impact of irradiation at doses eliminating foodborne pathogens on quality of apple fruit and orange juice, and to explore means of reducing or eliminating the adverse effects of irradiation.

EXPERIMENT ON APPLE FRUIT

The ripening of climacteric fruit, such as apple, is manipulated by the plant hormone, ethylene. The ethylene action inhibitor, 1-methylcyclopropene (MCP) (Sisler and Serek, 1997), slows apple fruit ripening including the loss of firmness and titratable acidity (Fan et al., 1999). It is unclear whether the irradiation-induced firmness and acidity loss can be eliminated or reduced by application of MCP. Many factors influence fruit responses to irradiation including fruit maturity, cultivar, storage temperature, and the use of controlled atmosphere storage (Maxie and Abdel-Kader, 1966; Miller and McDonald, 1999).

Although there are many reports that identify effects of irradiation on fruit texture, color, and other quality parameters (Massey et al., 1964; Maxie and Abdel-Kader, 1966; Willemot et al., 1996), information concerning impact of irradiation on the production of volatile compounds that contribute to aroma of whole fruit is limited. Volatile compounds are an important quality attribute of apple fruit. The most abundant volatile compounds produced by ripening apples are esters and alcohols.

MATERIALS AND METHODS

'Gala'apple (*Malus x domestica* Borkh.) fruit treated with 0.5 $\mu l\ l^{-1}$ MCP or air (non-MCP) for 12 h at 20 °C were exposed to gamma radiation at doses of 0, 0.44, 0.88, or 1.32 kGy at 23 °C using a GammaBeam 650 (Nordion International Inc, Kanata, Ontario, Canada) facility at dose rate of approximately 0.24 $kGy{\cdot}hr^{-1}$. The maximum/minimum dose ratio was 1.4. The description of the dosimetry system has been reported previously (Fan et al., 2001). The fruit were then stored at 20 °C for 3 weeks or at 0 °C for 8 weeks plus 7 days at 20 °C. Production of ethylene and other volatile compounds, respiration, fruit firmness, titratable acidity, soluble solids content, and irradiation damage were measured.

RESULTS AND DISCUSSION

Storage at 20 °C

During the 3 weeks post-irradiation storage at 20 °C, respiration rate of control fruit increased without delay (Fig. 1). One day after irradiation, respiration of non-MCP treated fruit was slightly stimulated by irradiation. Respiration was inhibited by irradiation at all doses 3 and 7 days after irradiation. In MCP-treated fruit, irradiation increased the respiration rate throughout the 21 days at 20 °C. Fruit irradiated with 1.5 kGy had highest respiration rate. Irradiation reduced ethylene production by non-MCP treated fruit in a dose-dependent manner (Fig. 1C,D). Irradiation of MCP-treated fruit resulted in a higher ethylene production 1 day after irradiation, but no irradiation effect was evident thereafter.

Ester production by non-irradiated control fruit increased to a maximum 7 days after the irradiation treatment. Treatment with MCP delayed the increase in ester production by 14 days and reduced the maximum rate attained over the 21-day period at 20 °C. Irradiation inhibited production of esters by both MCP-treated and control fruit (Fig. 2 A, B). The degree of inhibition increased with radiation dose.

The production of alcohols by control fruit reached a peak on day 7 (Fig. 2 C,D). MCP treatment or irradiation at doses of 0.88 and 1.32 kGy eliminated most of this increase. Alcohol production by non-MCP treated fruit irradiated at 0.88 kGy was higher than non-irradiated controls 1 and 3 days after irradiation but lower on days 7, 14 and 21. Non-MCP-treated fruit irradiated at 1.32 kGy also had lower alcohol production on days 7, 14 and 21 compared to non-irradiated controls. Irradiation at 1.32 kGy resulted in increased alcohol production by MCP-treated fruit 7 days after irradiation, all other irradiation treatments were similar to controls. MCP treatment generally inhibited production of volatile aldehydes and acetic acid throughout the 21-day period at 20 °C while irradiation reduced the production of aldehydes and acetic acid by control fruit only at day 7 when maximum productions were observed on non-irradiated control fruit (Fan et al., 2001).

After 21 days storage at 20 °C, compared to non-MCP treated fruit, fruit treated with MCP had higher firmness, soluble solids content (SSC), TA and internal injury and lower chroma (Table 1). The severity of internal injury increased with radiation dose. MCP increased sensitivity to irradiation. Although TA and firmness decreased with increased radiation dose in both MCP treated- and control- fruit, fruit treated with MCP followed by irradiation had higher firmness and TA than non-treated controls. SSC was not affected by irradiation except that control fruit irradiated with 0.88 kGy had the highest SSC. TA and chroma decreased with increased irradiation dose. The low chroma indicated that fruit were darker in peel color.

Storage at 0 °C

Similar to the effect on fruit stored at 20 °C, MCP decreased production of total volatile compounds, volatile esters and alcohols (Table 2). One day at 20 °C after 8 weeks storage at 0 °C, production of total volatile compounds, volatile esters and alcohols, decreased with radiation dose by both non-MCP treated and MCP-treated fruit. Aldehyde production was not affected by irradiation. After 7 days at 20 °C, irradiation decreased production of total volatile compounds, volatile esters, and alcohols in a dose depended manner in non-MCP treated fruit. Production of aldehydes was stimulated several fold by 1.32 kGy. Irradiation only reduced production of total volatile compounds and volatile esters by MCP-treated fruit. Fruit treated with 0.44 kGy had a similar production rate of total volatile compounds compared to nonirradiated and non-MCP treated controls after 8 weeks and 7 days at 20 °C.

After 8 weeks storage at 0 °C plus 7 days at 20 °C, MCP-treated fruit had higher firmness and TA, and similar SSC and chroma compared to controls (Table 2). Irradiation had no effect on firmness, SSC or chroma. TA decreased with increased radiation dose in both MCP-treated and non-MCP treated fruit. Irradiation caused little damage.

The contamination of apple juice/cider by *E. coli* O157:H7 may originate from contaminated fruit (Buchanan et al., 1999). The internalization of *E. coli* O157:H7 in apple fruit makes decontamination using chemical treatments impossible (Buchanan et al., 1999). Ionizing irradiation may provide a means of eliminating internal contamination because of its penetrating ability.

Our results indicate that irradiation at doses (300 Gray or less) sufficient to meet quarantine requirements did not significantly affect texture or soluble solids content of apple fruit, and only temporarily reduced production of some volatile compounds. Irradiation at doses reducing decay and inactivating 5-log *E. coli* O157:H7 promoted loss of firmness and acidity and reduced production of volatile compounds. Some responses of apple fruit to gamma radiation are influenced by ethylene action and post-irradiation storage temperature. Fruit treated with MCP followed by irradiation had higher firmness and TA compared to irradiated-fruit without MCP treatment, suggesting irradiation can be combined with MCP and proper storage temperature to maximize firmness and TA retention and minimize development of radiation damage.

Experiment on Orange juice

Orange juice is one of the largest sources of dietary Vitamin C (Fellers et al., 1990), and oxidation of Vitamin C in orange juice is related to changes in flavor and browning (Lee and Nagy 1988). Ascorbic acid (AA) can be converted to dehydroascorbic acid (DHA) under aerobic conditions, and DHA is further degraded to diketogulonic acid (Liao and Seib 1988). Both AA and DHA are biologically active while diketogulonic acid posses no activity. The effect of irradiation on the total "ascorbic acid" (TAA) (AA plus DHA) has not been fully investigated. Most reports (Proctor and O'Meara, 1951; Thakur and Arya, 1993) have dealt with sterilizing doses of irradiation and the impact of irradiation on AA and TAA at doses that inactivate the common human pathogens is unclear. The rates of degradation of AA and TAA during the post-irradiation storage period are not well described. Although off-odors have been observed in irradiated orange juice (Spoto et al., 1997; Thakur and Arya, 1993), the effect of irradiation on aroma volatile compounds of orange juice is unknown.

MATERIALS AND METHODS

Single-strength orange juice was irradiated at 0, 0.89, 2.24, 4.23 and 8.71 kGy at 5$\pm$ 2 °C using a ^{137}Cs gamma radiation source. Maximum/minimum dose ratio was 1.1. The custom-made, self-contained gamma-radiation source (Lockheed Georgia Company) has 23 137Cs pencils placed in an annular array around a 63.5-cm-high stainless steel cylindrical chamber with a 22.9-cm internal diameter. The source strength at the time of this study was ca. 109,159 Ci (4.039 PBq) with a dose rate of 0.10 kGy min-1. The dose rate was established using alanine transfer dosimeters from the National Institutes of Standards and Technology, Gaithersburg, MD. Corrections for source decay were made monthly. Routine dosimetry was performed using 5-mm-diameter alanine dosimeters (Bruker Instruments, Rjeomstettem, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR Analyzer (ASTM 1996). Variations in radiation dose absorption were minimized by placing the samples within a uniform area of the radiation field and by irradiating them within a polypropylene container (4-mm wall) to absorb Compton electrons and by using the same geometry for sample irradiation during the entire study. Alanine dosimeters were used to establish the absorbed dose at temperatures comparable to our calibration curve at 5°C.

Antioxidants, TAA, DHA, AA, and volatile compounds were measured during storage at 23 C (6 days) or 7 °C (21 days). Antioxidants were measured using the ferric reducing antioxidants power (FRAP) assay (Benzie and Strain 1996; 1999). TAA, AA, and DHA was measured using an HPLC method according to Doner and Hicks (1981), and volatile compounds, isolated by solid-phase microextraction, were separated and identified using a gas chromatograph equipped with a mass selective detector.

RESULTS AND DISCUSSION

Conversion of AA to DHA, and loss of AA, total AA (TAA) and total antioxidants increased with radiation dose (Figure 3). Loss of TAA caused by irradiation was much less than that of AA. Compared to non-irradiated juice, juices irradiated at 2.24 kGy or above had lower rates of AA loss during storage at 23 °C. The rate of AA loss during storage at 7 °C was, however, not altered by irradiation. Loss of total antioxidants in irradiated samples appeared to result from AA loss as the concentration of non-AA antioxidants was not affected by irradiation. Similar to the results with apple juice, irradiation did not affect pH or Brix, but reduced browning of juice (data not shown).

Variation in resistance to irradiation exists among different strains of *Salmonella*. *S. anatum* (D = 0.71kGy) was significantly more resistant than the other species tested (Niemira et al., 2001). *S. newport* (D = 0.48kGy) and *S. infantis* (D = 0.35kGy) were significantly different, while *S. stanley* (D = 0.38kGy) was intermediate between the two. At 3.55 kGy, sufficient for a 5-log inactivation of all *salmonella* strains, the estimated loss of TAA would be 16.6%. Other antioxidants, pH, and Brix in orange juice would not be affected significantly by the 5D dose of gamma radiation.

Fourteen volatile compounds were identified and quantified in orange juice (Table 3). Most of compounds were stable during storage at 7 °C while geranial and neral decreased. The most abundant compounds were ethanol and limonene. Irradiation did not affect the majority of volatile compounds, but reduced the amounts of geranial, neral, myrcene and linalool immediately after irradiation and after 1 week storage at 7 °C. The reduction increased linearly with radiation dose. The difference was, however, not significant after 3 weeks storage at 7 °C. Most volatile compounds in orange juice were terpenes, and it appears that only acyclic monoterpenes are sensitive to degradation by irradiation.

REFERENCES

1. American Society for Testing and Materials (ASTM). 1996. E16007-94 Standard practice for use of the alanine-EPR dosimetry system, 12.02:813-843. In 1996 Annual Book of ASTM standards. ASTM, West Conshohocken, Pa.
2. Benzie, I.F.F. and Strain, J.J. 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In *Methods in Enzymology*, Vol. 299, (L. Packer, ed.) pp. 15-27, Academic Press, New York.
3. Beuchat, L.R. 1996. Pathogenic microorganisms associated with fresh produce. J. Food Protect. 59, 204-216.
4. Buchanan, R.L., Edelson, S.G., Snipes, K. and Boyd, G. 1998. Inactivation of *Escherichia coli* O157:H7 in apple juice by irradiation. App. Environ. Microbiol. 64, 4533-4535.
5. Buchanan, R.L., S.G. Edelson, R.L. Miller, and G.M. Sapers. 1999. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. J. Food Protect. 62, 444-450.
6. CDC (centers for Disease Control and Prevention). 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider-Connecticut and New York, October 1996. Morbid. Mortal. Weekly Rep. 46, 1, 4-8.
7. Doner, L.W. and Hicks, K.B. 1981. High-performance liquid chromatographic separation of ascorbic acid, erythorbic acid, dehydroascorbic acid, dihydroerythorbic acid, diketogulonic acid, and diketogluconic acid. Ana. Biochem. *115*, 225-230.
8. Fan, X., Blankenship, S.M. and Mattheis, J.P. 1999. 1-Methylcyclopropene inhibits apple fruit ripening. J. Amer. Soc. Hortic. Sci. 124, 690-695
9. Fan, X., Argenta, A. and Mattheis, J.P. 2001. Impact of ionizing radiation on the volatile production of ripening 'Gala' apple fruit. J. Agric. Food Chem. 49(1): (in press).
10. Fellers, P.J., Nikdel, S. and Lee, H.S. 1990. Nutrient content and nutrition labeling of several processed Florida citrus juice products. J. Amer. Dietetic Assoc. 90, 1079-1084.

11. Hallman, G.J., 1999. Ionizing radiation quarantine treatments against tephritid fruit flies. Postharvest Biol. Technol. 16, 93-106.
12. Lee, H.S. and Nagy, S. 1988. Quality changes and nonenzymatic browning intermediates in grapefruit juice during storage, J. Food Sci. 53, 168-172.
13. Liao, M.L. and Seib, P.A. 1988. Chemistry of L-ascorbic acid related to foods. Food Chemistry. 30, 289-312
14. Massey L.M., Parsons, G.F. and Smock, R.M. 1964. Radiation processing of foods: Some effects of gamma radiation on the keeping quality of apples. J. Agric Food Chem. 12, 268-274.
15. Maxie, E.C. and Abdel-Kader, A., 1966. Food irradiation - Physiology of fruits as related to feasibility of the technology. Adv. Food Res. 15, 105-145.
16. Maxie, E.C., Sommer, N.F. and Mitchell, F.G. 1971. Infeasibility of irradiating fresh fruits and vegetables. HortScience 6, 202-204.
17. Miller, W.R. and McDonald, R.E. 1999. Irradiation, storage of maturity at harvest, and storage temperature during ripening affect papaya fruit quality. HortScience 24, 1112-1115.
18. Niemira, B.A., Sommers, C.H. and Boyd, G. 2001. Irradiation inactivation of iour *Salmonella* species in orange juices with varying turbidity. J. Food Protect (accepted).
19. Parish, M.E. 1998. Coliforms, *Escherichia coli* and *Salmonella serovars* associated with a citrus-processing facility implicated in a Salmonellosis outbreak. J. Food Protection. 61, 280-284
20. Proctor, B.E. and O'Meara, J.P. 1951. Effect of high-voltage cathode rays on ascorbic acid. In vitro and in situ experiments. Indus. Engin. Chem. 43, 718-721.
21. Sisler, E.C. and Serek, M. 1997. Inhibitors of ethylene responses in plants at the receptor level: Recent developments. Phys. Plant. 100, 577-582.
22. Spoto, M.H.F., Domarco, R.E., Walder, J.M.M., Scarminio, I.S. and Bruns, R.E. 1997. Sensory evaluation of orange juice concentrate as affected by irradiation and storage. J. Food Process Preserv. 21, 179-191.
23. Thakur, B.R. and Arya, S.S. 1993. Effect of sorbic acid on irradiation-induced sensory and chemical changes in sweetened orange juice and mango pulp. Internal. J. Food Sci. Technol. 28, 371-376.
24. Thayer, D.W., Rajkowski, K.T., 1999. Development in irradiation of fresh fruits and vegetables. Food Technol . 53, 62-65.
25. Thomas, P., 1986. Radiation preservation of foods of plant origin. Part V. Temperate fruits: Pome fruits, stone fruits, and berries. CRC Critical Rev. Food Sci. Nutri. 24, 357-400.
26. Willemot, C., M. Marcotte, L. Deschenes. 1996. Ionizing radiation for preservation of fruits. In: Processing Fruits: Science and Technology. Vol. 1. Biology, Principles, and Applications. (L.P. Somogyi, H.S. Ramaswamy, and Y.H. Hui, eds.). Technomic Publishing, Lancaster, PA pp. 221-260.

Table 1. Effect of Irradiation and MCP on Firmness, Soluble Solids Content (SSC), Titratable Acidity (TA), Surface Color (Chroma), and Internal Radiation Damage Quality of 'Gala'Apple. Fruit were Irradiated with 0, 0.44, 0.88, 1.32 kGy Gamma Radiation After Treated with Air (Control) or 0.5 $\mu L \cdot L^{-1}$ MCP. The Fruit were then Stored in Air for 3 Weeks at 20 °C

Dose (kGy)	Firmness (N)	SSC (%)	TA (%)	Chroma	Internal damage (1-4)
			Control fruit		
0	62.7	14.4	0.283	48.7	10
0.44	59.3	14.0	0.251	47.3	1.2
0.88	59.5	14.9	0.268	45.8	1.4
1.32	51.8	14.7	0.261	40.9	2.4
$LSD_{0.05}$	4.8	0.5	0.023	3.9	0.5
			MCP treated fruit		
0	83.3	14.9	0.321	42.7	1.0
0.44	75.3	14.8	0.297	42.8	1.6
0.88	72.3	14.7	0.284	33.6	2.0
1.32	61.7	14.8	0.281	32.6	3.1
$LSD_{0.05}$	4.5	0.36	0.021	5.1	0.4

Table 2. Effect of Irradiation and MCP on Firmness, Soluble Solids Content (SSC), Titratable Acidity (TA), Surface Color (Chroma), and Internal Radiation Damage Quality of 'Gala'Apple. Fruit were Irradiated with 0, 0.44, 0.88, 1.32 kGy Gamma Radiation After Treated with Air (Control) or 0.5 $\mu L \cdot L^{-1}$ MCP. The Fruit were then Stored in Air for 7 Weeks at 0 C plus 1 Week at 20°C.

Dose (kGy)	Firmness (N)	TA (%)	Internal damage (1-4)	Esters	Alcohols
			Control fruit		
0	64.2	0.265	1.0	2722.7	646.7
0.44	66.1	0.236	1.0	2174.1	710.9
0.88	62.3	0.238	1.0	389.0	404.1
1.32	63.2	0.243	1.1	201.0	219.6
$LSD_{0.05}$	5.3	0.025	0.1	216.1	137.0
			MCP treated fruit		
0	76.0	0.330	1.0	401.2	60.7
0.44	75.8	0.303	1.0	381.0	95.2
0.88	76.3	0.286	1.0	210.8	81.0
1.32	73.5	0.270	1.0	187.2	53.2
$LSD_{0.05}$	4.6	0.021		136.5	24.8

Table 3. Volatile Compounds of Orange Juice

Compound	Retention time (min)	Concentration (ppm)
Ethanol	1.32	513.33
Ethyl butyrate	2.23	0.73
ÿ-Pinene	3.67	1.46
ÿ-Pinene	4.15	0.05
Ethyl hexanoate	4.42	0.06
Myrcene*	4.48	5.09
Octanal	4.53	0.52
Limonene	5.03	131.94
Linalool*	6.07	3.26
ÿ-Terpineol	7.72	1.04
Decanal	7.86	1.88
Neral*	8.43	0.10
Geranial*	8.99	0.14
Valencene	12.59	3.92

* Compounds reduced by irradiation.

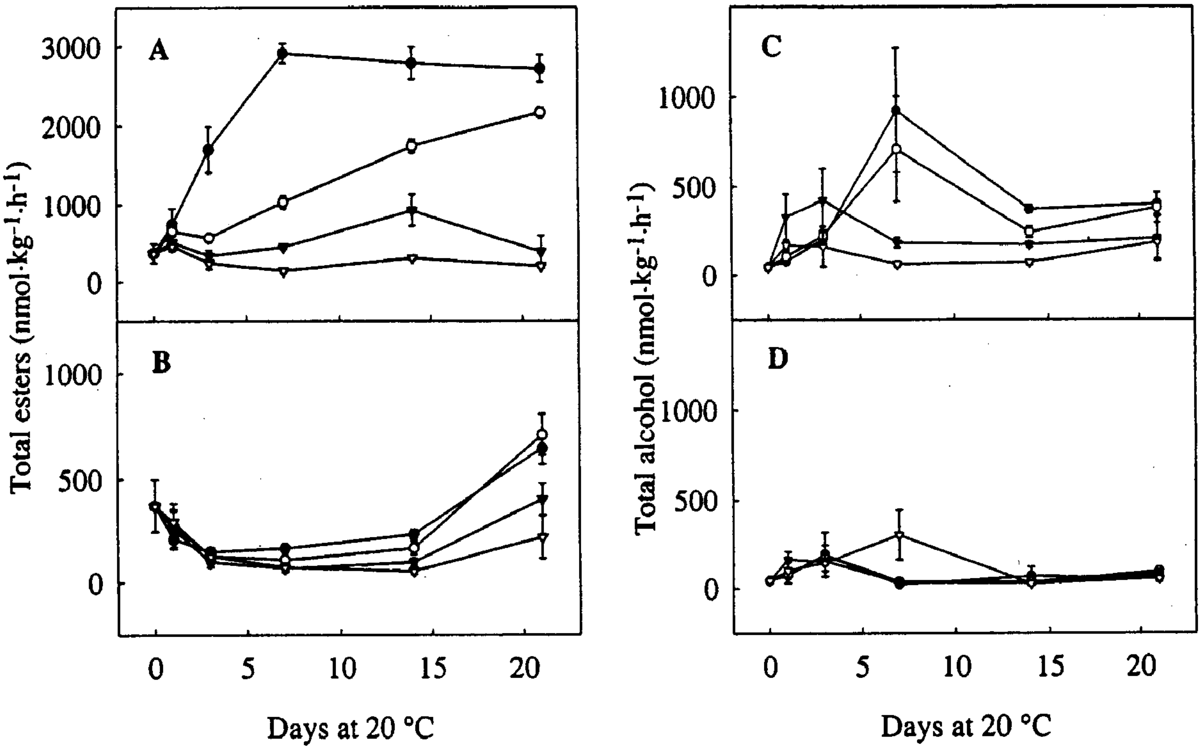

Figure 1. Production of ethylene (A, B) and respiration rate of (C, D) of 'Gala' apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88 and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

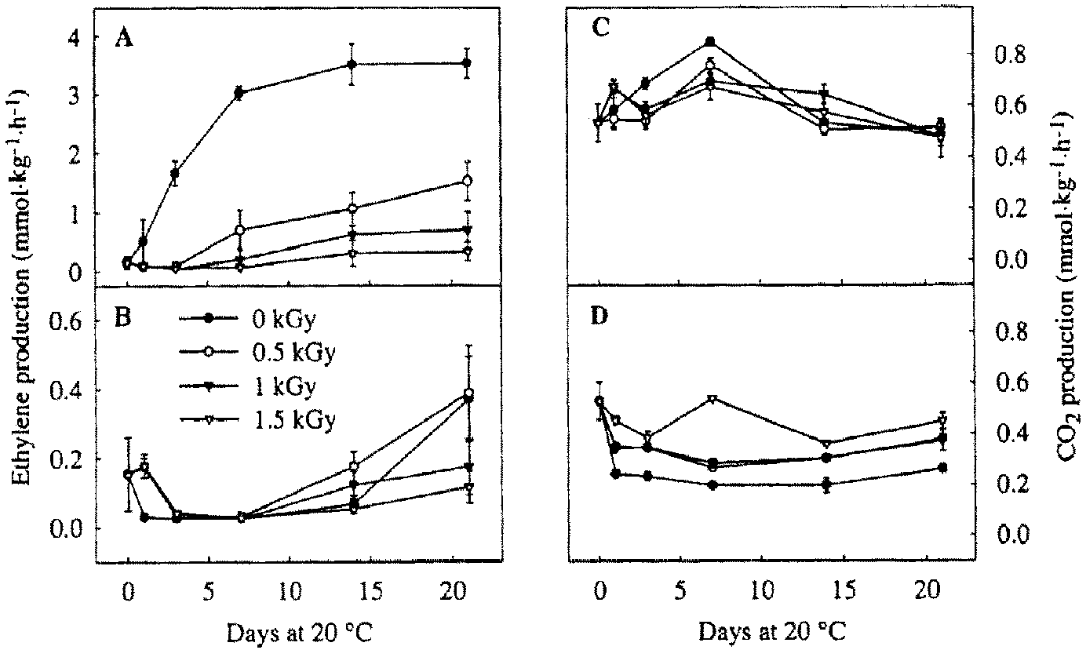

Figure 2. Production of total volatile esters (A, B) and alcohols (C,D) of 'Gala' apples during ripening. The fruit treated with (B, D) and without (A, C) 0.5 $\mu L \cdot L^{-1}$ MCP were exposed to 0, 0.44, 0.88 and 1.32 kGy gamma irradiation and kept at 20 °C. Vertical bars represent standard deviation.

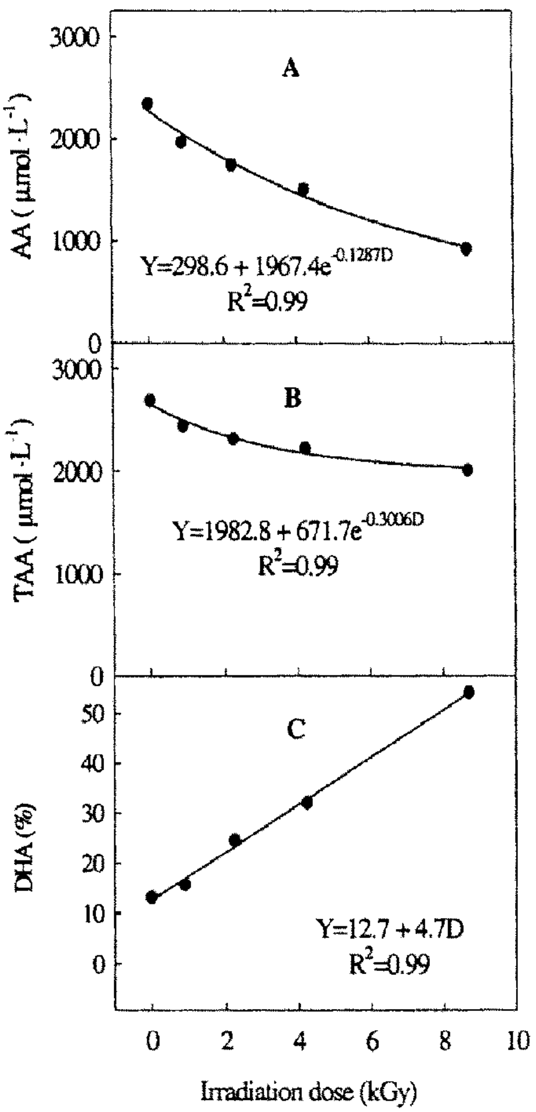

Figure 3. Content of ascorbic acid (AA) (A), total ascorbic acid (TAA) (B), and percentage of dehydroascorbic acid (DHA) in orange juice following irradiation. Juice was irradiated with gamma radiation at 0, 0.89, 2.24, 4.23 or 8.71 kGy at 5 C.